N-acetylcysteine and vitamin E: An *in vitro* study of their effect on homogentisic acid polymerization

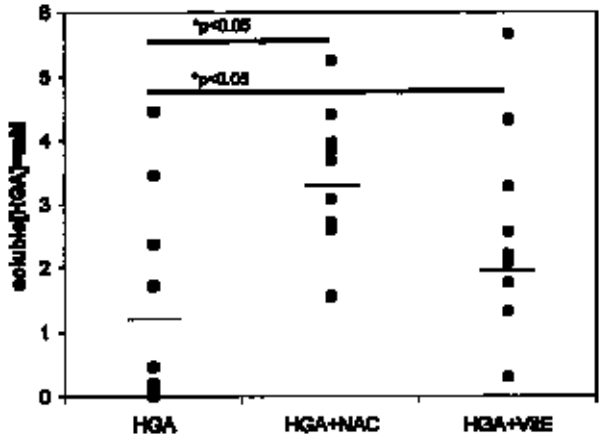

Fig. 1. Plasma concentration of non-polymerized homogentisic acid (HGA) after incubation at 37°C for 24 hours in the presence of *N*-acetylcysteine (NAC) or vitamin E (VitE). CG-MS analysis showed that the concentration of free HGA in the presence of *N*-acetylcysteine or vitamin E was significantly higher compared with the samples not treated with antioxidants.

Sirs,

Alcaptonuria is a rare genetic disease characterized by a deficiency of the enzyme homogentisic acid oxidase (HGA oxidase) (1). In the absence of HGA oxidase, HGA is rapidly oxidized to benzoquinoneacetic acid which then polymerizes to a melanin-like pigment (2-4). The aim of this study was to investigate the capacity *in vitro* of three well-known moieties with antioxidant properties, N-acetylcysteine (NAC), Vitamin E (Vit E) and *d*-α-tocopheryl polyethylene glycol 1000 succinate [a pegylated highly hydrophilic derivative of vitamin E (Vit E TPGS)] to inhibit the process of polymerization in plasma obtained from healthy subjects and supplemented with free HGA.

We studied 9 healthy subjects (5 male and 4 female) aged 30 ± 3 years (mean ± SD). Ten ml of heparinized blood were centrifuged at 500 g for 10 minutes. The plasma obtained was centrifuged at 1100 g for a further 10 minutes. Scalar concentrations of antioxidants (2.97, 5.94 and 11.89 mM) were tested against one concentration of HGA (5.94 mM). All samples were incubated at 37°C for 24 hrs. The degree of polymerization was evaluated by quantifying the amount of free HGA (5). Chemical analysis was carried out by means of gas chromatography and mass spectrometry analysis (GC-MS) (Hewlett-Packard model 5890 and Hewlett-Packard 5972 MS). Data were expressed as the mean ± SD unless otherwise indicated. Statistical analysis was carried out using ANOVA for repeated data and a multiple pair-wise comparison (Student-Newman-Keuls method) for comparisons between group means. P values < 0.05 were considered statistically significant.

A significant increase of free HGA in the presence of NAC and Vit E was detected by group comparison ($p = 0.00362$). Multiple pair-wise comparison showed a significant difference between the groups studied. The concentration of free HGA in the presence of NAC and Vit E was significantly higher compared to the samples not treated with antioxidants (3.22 mM ± 1.35 $p < 0.05$; 2.0 mM ± 1.59 $p < 0.05$; 1.29 mM ± 1.63 respectively; $n = 9$). The effect of NAC was stronger than that of Vit E,although the difference did not reach statistical significance ($p > 0.05$) (Fig. 1). Since NAC is more hydrosoluble than Vit E, we hypothesized that the anti-polymerizing potency of antioxidants could be related, at least in part, to their hydrosolubility. To investigate this we compared the action of liposoluble Vit E with that of Vit E TPGS on the HGA polymerization process. The results demonstrated that Vit E TPGS inhibited HGA polymerization more effectively than Vit E, although the difference did not statistical significance (Vit E TPGS: 2.48 mmM ± 2.184; Vit E: 1.8 mM ±1.12; $p = 0.08$, $n = 3$).

In this study we found evidence that NAC and Vit E are able to counteract *in vitro* the polymerization and accumulation of HGA, NAC being more effective than Vit E. The greater effectiveness of NAC is probably related at least in part to its better hydrophilic properties, since we observed a trend for a greater efficacy of Vit E TPGS in inhibiting HGA polymerization compared to standard liposoluble Vit E (6). Taking into account our results and previous data from the literature, it is conceivable that antioxidants could interfere with the pathogenic events believed to lead to the manifestations of alcaptonuria by means of different mechanisms. Firstly, they could act as scavengers of oxygen free radicals, thus limiting tissue damage. Secondly, as we have shown herein, they could prevent or delay the accumulation of HGA, the initial step in the sequence of abnormal reactions that take place in alcaptonuria. Finally, since NAC can neutralize the acetaminophen derivative N-acetylbenzoquinoneimine (7), whose chemical structure is similar to that of benzoquinoneacetic acid, it is tempting to speculate that a third mode of action of NAC could be a direct neutralization of benzoquinoneacetic acid.

Based on these considerations, we would suggest that prolonged treatment with NAC or Vit E could potentially be effective *in vivo* for preventing or delaying the course of ochronotic arthropathy.

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